Prohedonic properties of gamma-hydroxybutyrate are associated with changes in limbic resting-state functional connectivity

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Abstract: Objective Gamma-hydroxybutyrate (GHB) is an endogenous GHB-/GABA-B receptor agonist and a narcolepsy treatment. However, GHB is also abused for its prohedonic effects. On a neuronal level, it was shown that GHB increases regional cerebral blood flow in limbic areas such as the right anterior insula (rAI) and the anterior cingulate cortex (ACC). We aimed to further explore the association between the subjective and neuronal signatures of GHB. Method We assessed subjective effects and resting-state functional connectivity (rsFC) of an rAI- and an ACC-seed in 19 healthy male subjects after GHB (35 mg/kg p.o.) using a placebo-controlled, double-blind, randomized, cross-over functional magnet resonance imaging design. Results GHB increased subjective ratings for euphoria ($p < 0.001$) and sexual arousal ($p < 0.01$). Moreover, GHB increased rAI-rsFC to the right thalamus and the superior frontal gyrus and decreased ACC-rsFC to the bilateral paracentral lobule (all $p < 0.05$, cluster corrected). Moreover, GHB-induced euphoria was associated with rAI-rsFC to the superior frontal gyrus ($p < 0.05$, uncorrected). Conclusions GHB induces prohedonic effects such as euphoria and sexual arousal and in parallel modulates limbic rsFC with areas linked to regulation of mood, cognitive control, and sexual experience. These results further elucidate the drug's effects in neuropsychiatric disorders and as drug of abuse.

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Prohedonic properties of gamma-hydroxybutyrate are associated with changes in limbic resting-state functional connectivity

Short title: Limbic functional connectivity signature of GHB

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ABSTRACT

Objective: Gamma-hydroxybutyrate (GHB) is an endogenous GHB-/GABA-B receptor agonist and a narcolepsy treatment. However, GHB is also abused for its prohedonic effects. On a neuronal level it was shown that GHB increases regional cerebral blood flow in limbic areas such as the right anterior insula (rAI) and the anterior cingulate cortex (ACC). We aimed to further explore the association between the subjective and neuronal signatures of GHB.

Method: We assessed subjective effects and resting-state functional connectivity (rsFC) of an rAI-, and an ACC-seed in 19 healthy male subjects after GHB (35 mg/kg p.o.) using a placebo-controlled, double-blind, randomized, cross-over functional magnet resonance imaging design.

Results: GHB increased subjective ratings for euphoria ($p<.001$) and sexual arousal ($p<.01$). Moreover, GHB increased rAI-rsFC to the right thalamus and the superior frontal gyrus, and decreased ACC-rsFC to the bilateral paracentral lobule (all $p<0.05$, cluster-corrected). Moreover, GHB-induced euphoria was associated with rAI-rsFC to the superior frontal gyrus ($p<0.05$, uncorrected).

Conclusions: GHB induces prohedonic effects such as euphoria and sexual arousal and in parallel modulates limbic rsFC with areas that are linked to regulation of mood, cognitive control, and sexual experience. These results further elucidate the drug's effects in neuropsychiatric disorders and as drug of abuse.

Key words

Sodium oxybate, libido, anhedonia, liquid ecstasy, mania

1. INTRODUCTION

Gamma-hydroxybutyrate (GHB, or sodium oxybate) is an endogenous short chain fatty acid and gamma-aminobutyric acid (GABA) metabolite that occurs naturally in the mammalian brain, mainly in the hypothalamus, and basal ganglia (Bessman and Fishbein, 1963, Snead and Morley, 1981). Specific high affinity GHB binding-sites were first discovered in rat brain (Benavides et al., 1982), but are also found in humans and monkeys, predominantly in the hippocampus, neocortex (frontal, temporal, insula, cingulate, and entorhinal areas), and striatum (Castelli et al., 2000). GHB is considered a neurotransmitter (Snead, 2000). However, the physiological function of this putative neurotransmitter remained unresolved, with evidences pointing towards anti-apoptotic and neuroprotective activity (Wendt et al., 2014). Besides the high affinity agonism at GHB receptors, the molecule also binds with low affinity to GABA-B receptors, and most of its effects are mediated by activity at this receptor type when exogenously applied (Carter et al., 2009a). Moreover, GHB has strong downstream neuromodulatory effects also on glutamate, dopamine, serotonin, norepinephrine, and cholinergic transmission (Andresen et al., 2011).

As a medical drug, GHB is used for the treatment of neuropsychiatric disorders such as narcolepsy (Boscolo-Berto et al., 2012), alcohol withdrawal and relapse prevention (Leone et al., 2010), and fibromyalgia (Spaeth et al., 2013). Because of its unique pharmacological profile including mood enhancing, prosocial and prosexual effects the drug was also proposed as an experimental therapeutic in depression, especially with pronounced anhedonia (Bosch et al., 2012, Bosch and Seifritz, 2016, Mamelak, 2009), which is an important target of antidepressant treatment (Argyropoulos and Nutt, 2013). However, for the same prohedonic effects the drug is also instrumentalized as a drug of abuse (Miotto et al., 2001, Sumnall et al., 2008). As such, GHB is a very well-known substance among club drugs, as recently reported in different studies in samples of adolescents and young adults (Martinotti et al., 2015, 2017). In this population, GHB is frequently used in combination with other drugs such as alcohol, stimulants, and cannabis, which leads to more severe clinical intoxication features and more frequent need of acute medical treatment (Miro et al., 2017). The drug has an addictive potential that seems to strongly vary (0.04-21%) by the setting in which the drug is applied (medication vs. illicit use)(Carter et al., 2009b, Leone et al., 2010).

Although GHB was developed already 1960 by Henri Laborit (Kunz, 2014, Laborit et al., 1960), investigation of its neuronal effects using neuroimaging techniques started only recently. Here, we performed an experimental study on the subjective and neuronal effects of 35 mg/kg GHB p.o. in 20 healthy male participants. Due to the variety of used methods, produced results and covered topics, the investigation was split on four papers, focusing on task-related brain reactivity towards sexual stimulation (Bosch et al., 2017b), whole-brain resting-state cerebral perfusion and body and emotion awareness (Bosch et al., 2017a), whole-brain resting-state functional connectivity and vigilance effects (sedation and stimulation)(Bosch et al., 2018), as well as a seed-based analysis of the two most important GHB-related brain areas (rAI and ACC) and prohedonic effects (euphoria and sexual arousal), which we present here. We found that under influence of the drug, even sexually neutral pictures elicited sexual arousal and activated the bilateral nucleus accumbens (NAcc) and right anterior cingulate cortex (ACC) (Bosch et al., 2017b). Additionally, a psychophysiological interaction analysis revealed increased task-related functional connectivity between NAcc and ventromedial prefrontal cortex (vmPFC) during processing of erotic pictures under GHB, the condition in which subjective sexual arousal was highest (Bosch et al., 2017b). In a second analysis of the same sample, GHB increased body and emotion awareness and regional cerebral blood flow (rCBF) in limbic areas such as the right anterior insula (rAI) and the bilateral ACC , which correlated with each other (Bosch et al., 2017a). Moreover, a further resting-state functional connectivity (rsFC) analysis of these data showed that GHB increases salience network rsFC to the default mode network and dorsomedial prefrontal cortex (dmPFC), and from there reciprocally to the salience network (rIA) and to the central executive network (right middle frontal gyrus [MFG]). In this analysis, increased sedation significantly predicted the observed increase in rAI-dmPFC-rsFC (Bosch et al., 2018). Hence, first neuroimaging data in humans demonstrate GHB's activating effects on salience, reward, and central executive networks, with a key role for the rAI and the ACC.

To further explore the relationship between the neuronal signature on (within?) these structures and the prohedonic effects of the drug, we assessed hedonic subjective effects of GHB (35 mg/kg p.o.) including euphoria and sexual arousal (together with the typical side effect nausea), and the rsFC of the above mentioned rAI -, and ACC-seeding points (Bosch et al., 2017a) in a sample of 19 healthy male subjects that has been used for previous analyses (Bosch et al., 2017a, 2018). Similarities to the previous publications are the same study and sample. However, we did not use the results of previous functional connectivity analyses to guide any of the choices made

for the new functional connectivity analysis. This was particularly the case for the selection of the rAI and ACC seed regions in the seed-based functional connectivity analysis, which were not derived from the network-based indepentent component analysis (ICA) shown in the previous report (Bosch et al., 2018). In fact, ICA extracts intrinsic functional connectivity networks from the data without the need of a priori regions of interst (ROI). In contrast to previous reports, here we specifically targeted the whole-brain functional connectivity from a priori ROIs which we had found hyper-perfused by the GHB challenge using a different and completely independent data set (acquired using ASL-MRI (Bosch et al., 2017a). As we did not use the same data to define the seed ROIs and then to trace the connectivity, our results are free of the so called “double dip” issue (see, e.g., Kriegeskorte et al., 2009), i.e. we are not over-fitting the data. We used a placebo-controlled, double-blind, randomized, cross-over functional magnet resonance imaging (fMRI) design with four time points for rsFC assessment (baseline, and 34, 59 and 79 minutes post GHB intake) and hypothesized that GHB would alter rAI- and ACC-rsFC. Moreover, we expected that changes in limbic rsFC are correlated with the hedonic subjective effects of the drug.

2. METHODS

2.1 Design and Participants

A randomized, double-blind, placebo-controlled, balanced, crossed within-subject design was employed in 19 non-smoking healthy male subjects with a mean age of 23.5 years (standard deviation ±3.6, range, 20 - 36), a mean verbal intelligence quotient (IQ) of 113.4 (±18.4, 88 - 145), and a mean weight of 72.2 kg (±7.4, 59 - 85 kg). Subjects were recruited by online advertisings and underwent a medical and psychiatric examination applying the Structured Clinical Interview for DSM-IV Axis-I Disorders (First et al., 2002). Exclusion criteria were any DSM-IV psychiatric disorder, neurological disorder, severe medical disease, left-handedness, and regular illegal drug use (lifetime use on five and more occasions, with exception of occasional cannabis use), the latter was assessed by using the Interview for Psychotropic Drug Consumption (Quednow et al., 2004). Participants performed a German vocabulary test, the Mehrfachwahl-Wortschatz-Intelligenztest (Lehrl, 2005), to estimate the verbal intelligence quotient. They had to abstain from drinking alcohol 24 h before the experimental sessions and from drinking caffeinated beverages during the course of the study days. Abstinence from illegal drugs at the test sessions was ensured by semi-quantitative drug urine tests (Dimension RXL Max, Siemens, Erlangen, Germany). The study was approved by the Cantonal Ethics Committee of Zurich and by Swissmedic, and was registered at ClinicalTrials.gov (NCT02342366). All participants gave written informed consent and were financially compensated.

2.2 Procedure

GHB and placebo were applied in two sessions separated by seven days. On both test days, participants completed an fMRI paradigm (Bosch et al., 2017b) on a Philips Achieva 3 Tesla whole-body MR-unit equipped with a 32-channel receive head coil (Philips Medical Systems, Best, The Netherlands). The experiment started with a T1-weighted anatomical brain scan, a baseline rsFC scan, and an arterial spin labeling (ASL) scan. Subsequently, participants were taken out of the scanner and were orally administered with a single dose of GHB (35 mg/kg) or placebo (t=0 min). As C_{max} of GHB can be expected after about 40 min (Liechti et al., 2016), the fMRI paradigm began at t +30 min. Then, the procedure of scans was as follows: rsFC (34 min), ASL, task scan, rsFC (59 min), ASL, task scan, rsFC (79 min), ASL. Subjective drug effects were assessed using visual analogue scales (VAS) (100mm scale, with item name and according statement, e.g. 'I feel euphoric' for euphoria, with 'not at all' and 'strongly' on

the poles of the scale, selection via hand tool with scroll function inside the scanner, between scans) on *general drug effect, sedation, relaxation, stimulation, euphoria, body awareness, emotion awareness, sexual arousal, dizziness*, and *nausea.* The VAS scales were applied at time points t= -25 min before and 45, 55, 68, 77 and 93 min after drug/placebo administration. Experimental sessions lasted 200 min. Here, the GHB effects on *euphoria, sexual arousal*, and *nausea* as well as rsFC data of the rAI- and ACC-seed analyses are presented, while other fMRI data have been published elsewhere (Bosch et al., 2017a,b, 2018).

2.3 MRI Data Acquisition

The rsFC time series were acquired with a sensitivity-encoded single-shot echo-planar imaging sequence (SENSE-sshEPI, Schmidt et al., 2005). The rsFC protocol employed the following acquisition parameters: TE=35 ms, TR=2000 ms, flip angle=82°, FOV=22 cm^2, acquisition matrix=80x80 (in plane voxel size=2.75x2.75 mm^2), 32 contiguous axial slices (placed along the anterior-posterior commissure plane) with a slice thickness of 4 mm, and a SENSE factor R=2.0. For structural reference, a magnetization prepared rapid gradient-echo (MP-RAGE) T1-weighted anatomical scan was acquired with the following parameters: voxel size= 1x1x1 mm3, time between two inversion pulses= 2180 ms, inversion time= 610 ms, inter-echo delay= 9.3 ms, flip angle= 8°, 160 sagittal slices, FOV 240×240 mm^2, voxel size=1×1×1 mm^3.

2.4 MRI Data Preprocessing

Standard image data preparation and pre-processing, as well as statistical analysis and visualization were performed with the software BrainVoyager QX (Brain Innovation BV, The Netherlands). Functional data preprocessing included a correction for slice scan timing acquisition, a 3 dimensional rigid body motion correction, a spatial smoothing (gaussian kernel of 6 mm full-width-half-maximum), a temporal high-pass filter with cut-off set to 0.008 Hz per time-course and a temporal low-pass filter (Gaussian kernel of 3 s).

Structural and functional data were co-registered and spatially normalized to the Talairach standard space using a twelve parameter affine transformation. In the course of this procedure, the functional images were resampled to an isometric 3x3x3 mm^3 grid covering the entire Talairach box. Nuisance signals (white matter and cerebro-spinal fluid signals) were regressed out from each data set together with motion translation and rotation estimates after segmenting the entire brain, the white matter and ventricles from the normalized T1 volume.

A seed-based analysis was performed to study rsFC from the rAI and the ACC according to GHB-induced rCBF increases in a previous study (Bosch et al., 2017a). To compute rsFC maps corresponding to a selected seed region of interest (ROI), the mean regional time course was extracted from all ROI voxels and correlated against all voxels of the brain. The definition of two ROIs was based on our previous work (Bosch et al., 2017a) and separate correlation maps were produced for each subject, condition and ROI. The correlation maps were applied the Fisher's r-to-z transform $z= 0.5 \text{ Ln } [(1+r)/(1-r)]$ before entering the second-level random-effects statistical analysis.

2.5 Statistical Analysis of VAS Data

For the analyses of VAS scales, repeated measures analyses of variance (ANOVA) with drug (2-fold: GHB, placebo) and time (6-fold) as within-subject factors were applied using SPSS®22.0 for Windows. Greenhouse-Geisser correction and adjusted p-values were used in models with more than one degree of freedom in the numerator. Bonferroni-corrected paired t-tests were applied for post hoc treatment comparisons (placebo vs. GHB, 6 time points). All confirmatory statistical comparisons were carried out at a significance level of $p<.05$ (two-tailed).

2.6 Statistical Analysis of MR Images

For the whole-brain voxel-based rsFC analysis, all Talairach-normalized rsFC z-maps from the preprocessed 144 rsfMRI data sets (18 subjects [one subject was excluded from these analyses due to missing data in one of the sessions], 2 sessions, 4 repeated scans) were combined and entered into the analysis of covariance (ANCOVA) module of BrainVoyager QX. Here, a 3-way (4x2x2) mixed-effects ANOVA design was specified with two within-subject factors (*scan*, *treatment*), and one between-subject factor (*session*). Following the experimental design, the factor *scan* was assigned with four levels (baseline, 34 min, 59 min, 79 min), the factor *treatment* with two levels (GHB, placebo) and the factor *session* had two levels (GHB-first, placebo-first). All interactions between and among all three factors were also added to the model.

After least square model fitting, to detect any effects of systematic rsFC changes in relation to time, treatment and session, the F statistics for the 3-way interaction (scan x treatment x session) was computed at each voxel, yielding a whole-brain F-map, which was overlaid in pseudo-color onto the average normalized T1 image. To protect against false positives and correct for multiple comparisons, only statistically significant regional effects were displayed for compact clusters surviving the joint application of a voxel- and a cluster-level threshold, which were chosen using a non-parametric randomization approach based on Monte Carlo simulations. Namely, an arbitrary (uncorrected) threshold ($p< .005$ oder 0.05?) was initially applied to all voxels; then, a minimum cluster size was set in such a way that an average of 5% false positive clusters were counted in 1000 randomly generated images to which the same thresholds were applied. To match the level of *smoothness* between the calculated F-map and the simulated

images, after random number generation at each voxel, the resulting images were spatially filtered with a Gaussian kernel at the full width at half maximum initially estimated from the data according to (Forman et al., 1995).

For regions identified in the above analysis, mean regional functional rsFC z-values were extracted for each scan, session and subject, and used for ROI based correlation analyses with subjective VAS measures *euphoria*, *sexual arousal*, and *nausea*. Correlation analyses entailed with calculating the regional rsFC z value and VAS changes between a given experimental point (34, 59, 79 min) and baseline (Δ_Z and Δ_VAS), linearly regressing Δ_VAS against Δ_Z values in each separate treatment (GHB or placebo) and linearly covarying (ANCOVA) Δ_VAS and Δ_Z between separate session (GHB vs. placebo). Linear regression analyses produced coefficient of determination (R^2) separately for the two treatments. ANCOVA analyses were added to estimate the statistical significance of the change in the slope of the regression line between GHB and placebo sessions (interaction between rsFC Z value correlation and treatment).

3. RESULTS

3.1 Subjective Ratings

In drug × time (2×6) ANOVAs, the euphoria and sexual arousal VAS ratings showed significant time ($F(1,15)=6.05$ - 18.37, $p<.001$) and drug ($F(5,11)=10.26$ - 16.74, $p<.01$ - .001) effects and a drug × time interaction for euphoria ($F(5,11)=7.81$, $p<.001$), while only a trend drug effect was revealed for nausea ($F(5,11)=3.96$, $p=.065$). Paired t-test (Bonferroni-corrected) revealed significant GHB effects for increased euphoria between 45 and 68 min and for enhanced sexual arousal at 45, 68 and 93 min after drug intake. In contrast, even an exploratory post hoc analysis of the nausea VAS did not show significant drug effects at any time point (**Figure 1**). Of note, the two peaks in sexual arousal at 55 and 77 min after intake (Figure 1B) are explained by the fMRI task that was presented before, in which erotic pictures were shown (Bosch et al 2017b).

- Figure 1 -

3.2 Neuroimaging

We performed a seed-based analysis for possible 3-way interaction effects in a voxel-based 3-way ANOVA analysis with factors scan (baseline, 34, 59, 79 min after intake), treatment (GHB, placebo), session (GHB-first, placebo-first), using the rAI as well as the ACC according to a previous study (Bosch et al., 2017a). We found a significantly increased rsFC of the rAI seed with the bilateral superior frontal gyrus and with the right thalamus (both $p<.05$, cluster corrected) (**Figure 2**). In contrast, ACC-rsFC was decreased to the bilateral paracentral lobule ($p<.05$, cluster corrected) (**Figure 3**).

For all significant rsFC, changes in the subjective effects (e.g. Δ_VAS = VAS1 vs. VAS2) were correlated with the corresponding change in the RT-FC values (Δ_Z) after GHB challenge. The ANCOVA analysis highlighted a statistically significant ($p<.05$) correlation between Δ_Z (34 min) and Δ_VAS (45 min) for euphoria at including the slope effect (i.e., the interaction) with the bilateral superior frontal gyrus (Figure 4).

-Figures 2-4-

4. DISCUSSION

Here, we investigated the neural effects of the mixed GHB-/GABA-B receptor agonist GHB using rsFC measures of two seeds, namely the rAI and ACC, which were previously shown to exhibit increased rCBF under influence of this drug (Bosch et al., 2017a). The presented results are part of a study that was published in three other papers (Bosch et al., 2017a,b, 2018). We found that in healthy male subjects, 35 mg/kg p.o. GHB elicits hedonic subjective effects such as euphoria and sexual arousal, but not nausea. At the neuronal level, rsFC of the rAI increased to the bilateral superior frontal gyrus and the right thalamus. Moreover, rsFC of the ACC decreased to the bilateral paracentral lobule. Finally, increased rAI-rsFC to the bilateral superior frontal gyrus was correlated with euphoria indicating that the strengthened connectivity between the anterior insula region and the dorsolateral prefrontal cortex might account for the euphorogenic effects of GHB. These results are in accordance with the clinical data in narcolepsy and addiction. However, it is important to emphasize that our results were generated in healthy subjects and that therefore no direct clinical conclusions should be drawn.

Anhedonia, defined as an impaired ability to experience feelings of pleasure with previously rewarding stimuli, is one of the core symptoms of major depressive disorder (APA, 2013). Occurrence of anhedonia and associated depression symptoms such as feelings of emptiness or dysphoria, loss of appetite and sexual arousal, as well as social withdrawal, are understood to reflect impairments of neural salience and reward networks (Heshmati and Russo, 2015, Russo and Nestler, 2013). These involve cortical structures such as the rAI, ventro- and dorsomedial prefrontal cortex (vmPFC, dmPFC), and the ACC, as well as subcortical structures such as the NAcc, the thalamus, and the ventral tegmental area (VTA) (Sternat and Katzman, 2016). In the same sample, we have recently shown that GHB increases the activity in neural salience and reward networks, which was associated with hedonic subjective effects such as sexual arousal during and after visual stimulation (ACC, NAcc, vmPFC) and increased body and emotion awareness (rAI, ACC) (Bosch et al., 2017a,b), as well as sedation (salience network-dmPFC rsFC). However, these network effects are also discussed as the mechanisms of the addictive potential of GHB (Abanades et al., 2007, Carter et al., 2006, 2009a,b).

From a psychopharmacological perspective it is remarkable that a predominant GABA-B agonist exerts a spectrum of subjective effects that is usually attributed either to tranquilizers (sedation, relaxation, anxiolysis) or stimulants

(euphoria, stimulation, sexual arousal) (Abanades et al., 2006, 2007, Barker et al., 2007, Bosch et al., 2015, 2017b, Kapitany-Foveny et al., 2015, Miotto et al., 2001, Sumnall et al., 2008). Here, we show that GHB induces euphoria and sexual arousal. Euphoria peaked at 45 minutes and vanished to placebo level at 77 minutes post drug intake. On the other hand, for sexual arousal the most significant elevation in the GHB group compared to placebo was observed also at 45 minutes, with two more significant time points at 68 and 93 minutes. Quantitatively, there were two peaks at 55 and 77 minutes due to visual stimulation, were sexual arousal was increased in both the GHB and the placebo group, resulting in no significant difference between the groups. Both profiles are in line with previous pharmacokinetic and pharmacodynamic studies, in which GHB had a t_{max} and half-life of approximately 40 minutes, and induced typical subjective effects such as sedation, stimulation, and dizziness peaking at 40 minutes and vanishing at 100 to 180 minutes post intake (Abanades et al., 2006, Bosch et al., 2015, Brenneisen et al., 2004, Liechti et al., 2016). Depending on dosage and time, GHB frequently exerts biphasic effects such as euphoria in lower doses and in early stages of intoxication and sedation or coma with higher doses and in later stages of intoxication (Abanades et al., 2006, Madah-Amiri et al., 2017). In our studies, we did not find differences in peak times regarding hedonic effects such as euphoria and sexual stimulation (present data), or vigilance effects such as sedation and stimulation (Bosch et al., 2015, 2018). However, in data from the same study published elsewhere (Bosch et al., 2018) and from an earlier study (Bosch et al., 2015), sedative GHB effects lasted significantly longer then stimulating effects. It was proposed that these differences might be attributable to divergent dose-dependent receptor activation patterns (Bosch et al., 2015). While stimulation and euphoria seem to be mediated by a short-lasting disinhibition of thalamocortical neurons and neurons in the VTA via agonism at presynaptic GABA-B receptors, sedative effects involve inhibition of these neurons via post synaptic GABA-B receptors, as well as G protein-gated inwardly rectifying potassium (GIRK) channels, leading to a delayed and more intense neuronal hyperpolarization (Luscher and Slesinger, 2010).

Euphoria and sexual arousal are frequently reported by illicit users of GHB (Kapitany-Foveny et al., 2015, Sumnall et al., 2008), but the specificity of these effects is still a matter of debate. These hedonic subjective effects represent the main reasons for the illicit instrumentalization of GHB (Luby et al., 1992, Sumnall et al., 2008) and they are also proposed as potentially therapeutic features in psychiatric conditions with occurrence of anhedonia (Bosch and

Seifritz, 2016). Both, euphoria and sexual arousal are linked to frontolimbic dopaminergic activity (Drevets et al., 2001, Wise and Bozarth, 1985), which is dampened in certain anhedonic states (Argyropoulos and Nutt, 2013). In fact, although most of the subjective and behavioral actions of GHB are mediated by its GABA-B agonism (Carter et al., 2009a), its specific hedonic properties such as mood enhancement, as well as prosocial and prosexual activation might be mediated by downstream dopaminergic disinhibition of the VTA via GABAergic autoreceptors on GABAergic interneurons (Bosch et al., 2015, 2017a,b, Cruz et al., 2004, Labouebe et al., 2007, Maitre, 1997, Pistis et al., 2005). Although GHB did not induce significant nausea in our volunteers at 35 mg/kg p.o., this is usually a typical side effect of high doses of GHB (Carter et al., 2006, Chin et al., 1992, Elsing et al., 2009, Russell et al., 2011), which is probably also related to the drug's capacity to rapidly increase central dopaminergic tone. However, the same dose robustly induced dizziness in our participants (Bosch et al., 2017a), which is also a cardinal symptom of GHB intoxication (Bosch and Seifritz, 2016).

On a neuronal level, we aimed to further explore GHB-induced limbic activation in the rAI and the ACC, as both structures proved to be the core hubs of GHB effects in the human brain, assessed by neuroimaging techniques. In our study of which the here presented data is one part, we found that sexual arousal under GHB and visual stimulation involves task-related processing via ACC, Nacc, and vmPFC (Bosch et al., 2017b), increase of salience network/rAI-dmPFC rsFC which predicted sedation (Bosch et al., 2018), and resting state rCBF increase in the rAI and the ACC, both associated with increased body and emotion awareness (Bosch et al., 2017a). Consequently, we decided to use the rAI and ACC as seed regions in relationship to prohedonic effects to increase the neuropsychopharmacological knowledge about GHB-related limbic mechanisms.

There is strong evidence from previous neuroimaging studies that the rAI is not only a core hub of the salience network, but that it is also selectively activated during the experience of intense positive feelings including interoceptive components (Craig, 2009). In a positron emission tomography investigation of enhanced mood after physical activity, euphoria was correlated with activity in the rAI and the ACC, but also the right superior frontal gyrus (Boecker et al., 2008), latter of which is the area in which we found a correlation of increased rAI-rsFC and euphoria. Picard and colleagues (2013) demonstrated that direct electrical stimulation of the rAI in an epilepsy patient triggered typical ecstatic auras with an intense feeling of bliss including pleasurable body sensations (Picard

et al., 2013). Using the above mentioned rAI coordinates as seed area for an rsFC analysis, here we found that GHB increases rsFC of the rAI to the bilateral superior frontal gyrus, which is part of the central executive network, and the right thalamus. Increased rAI rsFC to the superior frontal gyrus was correlated with euphoria. In a previous rsFC network analysis of the same study, salience network/rAI rsFC was increased to the default mode network and to the dmPFC, and rAI-dmPFC rsFC predicted increased sedation (Bosch et al., 2018). Moreover, increased rAI rCBF was associated with body and emotion awareness (Bosch et al., 2017a). Consequently, these results add new insights to the significance of the rAI as a core element of the neuronal mediation of GHB effects in humans. More important, it clarifies that GHB has major impact on the salience network and its consecutive recruitment of areas belonging to the central executive network (MFG) and that this is a mechanism by which euphoria, one of the drug's stimulant-like effects, is mediated. Regarding recently discovered rAI-rsFC reductions to the central executive network in major depression (Iwabuchi et al., 2014), this GHB mechanism might be of high relevance for a biomarker-driven treatment of affective disorders.

The other brain area that showed increased rAI-rsFC in our sample was the right anterior thalamus. This subcortical structure is anatomically connected to the ACC, frontal cortex, and insula via afferent neuronal projections and involved in the processing of emotional, somatosensory, and executive functions (Klein et al., 2010, Lane et al., 1997, O'Muircheartaigh et al., 2015, Price, 2000, Sandson et al., 1991). From a phenomenological perspective it is noteworthy that right thalamic lesions are sometimes associated with mania-like symptoms such as euphoria and disinhibition (Bogousslavsky et al., 1988, Kulisevsky et al., 1993), which were both also described for electrical stimulation of the rAI (Picard et al., 2013), and under influence of GHB (Bosch et al., 2015). On a neuronal level, it is well known that the thalamus has an important bottom-up gating and regulating function on cortical activity (Cappe et al., 2012), and contributes to the salience and the reward networks (Cho et al., 2013, Menon and Uddin, 2010, Menon, 2015). As GHB induces absence-like electrophysiological patterns in some mammals, the effects of GHB on thalamocortical projections were extensively studied (Venzi et al., 2015). In this context, in vitro studies using cat and rat thalamus have revealed differential pre- and postsynaptic effects of GHB on thalamocortical neurons. Postsynaptically, GHB applied to thalamic GABA-A neurons indirectly increased tonic inhibition (Cope et al., 2009), resulting in decreased cortical activity. Also presynaptically, GHB-induced activation of GABA-B receptors

reduces sensory and thalamocortical excitatory postsynaptic potentials (EPSPs) (Emri et al., 1996, Gervasi et al., 2003). However, GABA-B autoreceptors on GABAergic neurons also have an important function for thalamocortical disinhibition, as GHB reduces somatosensory inhibitory postsynaptic potentials (IPSPs) via this mechanism (Gervasi et al., 2003), leading to increased cortical activity. Other studies also strongly point to the disinhibiting role of GABA-B receptors for thalamocortical projections (Chen and van den Pol, 1998, Le Feuvre et al., 1997, Mouginot et al., 1998, Ulrich and Huguenard, 1996a, b). Thus, on the thalamic level GHB seems to work as a differential regulator of cortical activity. Consequently, the observed increase of rAI-thalamic rsFC is most likely generated by GABA-B mediated disinhibition of somatosensory projections, which is the bottom-up element of an overall activation of the salience and reward networks, corresponding to subjective hedonic effects such as euphoria, sexual arousal, and enhanced body and emotion awareness (Bosch et al., 2015, 2017a,b).

Another limbic area that was explored in this study is the ACC, which is a further key structure for the mediation of the neuropsychopharmacological effects of GHB. Activation of a salience and reward network including the ACC, vmPFC, and NAcc – presumably due to mesolimbic dopamine disinhibition via the VTA – was shown to be the neural underpinning of GHB-induced prosexual effects (Bosch et al., 2017b). This strengthens previous findings that the ACC mediates motivational aspects of behavior, including sexual arousal, by modulating reward processing through its projections to the NAcc (Sesack and Grace, 2010, Sewards and Sewards, 2003). Moreover, GHB increased ACC rCBF, which was the reason to investigate the rsFC of this area in the here presented analysis (Bosch et al., 2017a). In our subjects, we found a reduction of ACC-rsFC to the paracentral lobule. This is was in line with our expectations, as the paracentral lobule is a core hub of the somatosensory network and involved in the processing of body perception (Lavagnino et al., 2014), pain (Roy et al., 2009, Seminowicz and Davis, 2007), and sexual experience (Moulier et al., 2006), again functions on which GHB exerts considerable effects (Bosch and Seifritz, 2016). Interestingly, paracentral lobule rsFC was associated with psychopathology in bulimia nervosa such as impaired food intake behavior and body awareness (Lavagnino et al., 2014). On the other hand, GHB was shown to be effective in normalizing food intake behavior in binge eating syndrome (McElroy et al., 2011), and altering body awareness via rCBF increase in the here investigated ACC region (Bosch et al., 2017a). Moreover, in healthy subjects the perception of pain was associated with increased activity in the paracentral lobule (Roy et al., 2009,

Seminowicz and Davis, 2007), while on the other hand, GHB reduces pain in patients with fibromyalgia (Russell et al., 2009, 2011, Scharf et al., 2003). Regarding the topography of the somatosensory cortex, the paracentral lobe represents the genitals in females and males (Komisaruk et al., 2011, Narici et al., 1991). Clitoral, vaginal, and cervical self-stimulation all activated medial paracentral lobule in a sensory cortex mapping study (Komisaruk et al., 2011). Even imagined genital self-stimulation resulted in increased activity in this area (Wise et al., 2016). In males, partial seizures affecting the paracentral lobule were reported to be associated with genital sensations and penile erection (Stoffels et al., 1980). Moreover, in a study with healthy male volunteers, penile erection evoked by erotic pictures correlated with activation of a canonic sexual arousal network including the paracentral lobule (Moulier et al., 2006). Both the paracentral lobule and the ACC have important roles in the mediation of sexual cue-reactivity, sexual arousal, and physical sexual experience. However, while the ACC seems to exert rather top-down, motivational, and controlling functions, the paracentral lobule processes the physical, i.e., genital aspect of sexual experience. While rsFC increases tend to represent recruitment of brain areas to other areas or networks, rsFC-reductions are either interpreted as reduced function or exclusion of a certain area or – in case of top-down structures – a reduction of control and thus a disinhibition of the target area. Latter mechanism seems to explain best the here found ACC-rsFC reduction to the paracentral lobule. Finally, although we found a correlation between ACC activity and sexual arousal in our previous analysis (Bosch et al., 2017b), no such association of ACC-rsFC with the paracentral lobule could be detected here. This lack of association may have various reasons. Still, most likely either there is no association of ACC-paracentral lobule-rsFC with sexual arousal, or our study had not enough power to detect it. An evidence for the second option might be that nausea, although being a typical side effect of the here used doses of GHB, did only occur on a trend level (two subjects developed clinically relevant nausea under GHB, but the group comparison was not significant). Moreover, in the previous analysis (Bosch et al., 2017b), significant neuronal and prosexual effects were related to active visual stimulation, while the here presented analysis focused on the overall and rather intrinsic prosexual effects of GHB, which are less intense. Still, GHB clearly induced hedonic subjective effects and increased limbic rsFC to areas that are linked to these phenomena. A limitation of our study is that effectivity of blinding was not systematically assessed, and therefore is questionable due to recognizable subjective drug effects. Another potential limitation of our study is that part of the data had been published previously (Bosch et al., 2017a,b, 2018), thereby reducing the actual researcher degrees of

freedom, although none of the new results were obtained by introducing circularities in the analyses. At this level, our data do not allow direct clinical conclusions, but offer new hypotheses regarding potential new clinical applications of GHB. As the subjective and neuronal conditions in healthy subjects and patients are different, the here found promising properties should be further investigated in patients suffering from anhedonia and/or dysfunctions in the salience and reward networks such as major depressive disorder, in order to develop biomarker-driven treatment opportunities and enhance clinical outcome. xx

5. CONCLUSION

In summary, as expected GHB exerts hedonic subjective effects such as euphoria and sexual arousal, which are paralleled by increased rsFC of the rAI to the bilateral superior frontal cortex and the right thalamus, and decreased rsFC of the ACC to the paracentral lobule. Moreover, increased rAI-rsFC to the bilateral superior frontal cortex correlated with GHB-induced euphoria. Consequently, GHB modulates limbic rsFC with areas that are linked to regulation of mood, cognitive control, and sexual experience, further explaining the drug's unique acute effects at the neuronal level.

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Declaration of Conflicting Interests

The Authors declare that there is no conflict of interest.

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Figure Legends

Fig. 1: Effects on visual analogue scale (VAS) scores of GHB vs. placebo for *euphoria*, *sexual arousal*, and *nausea*. ***p<.001, **p<.01, *p<.05 (Bonferroni-corrected).

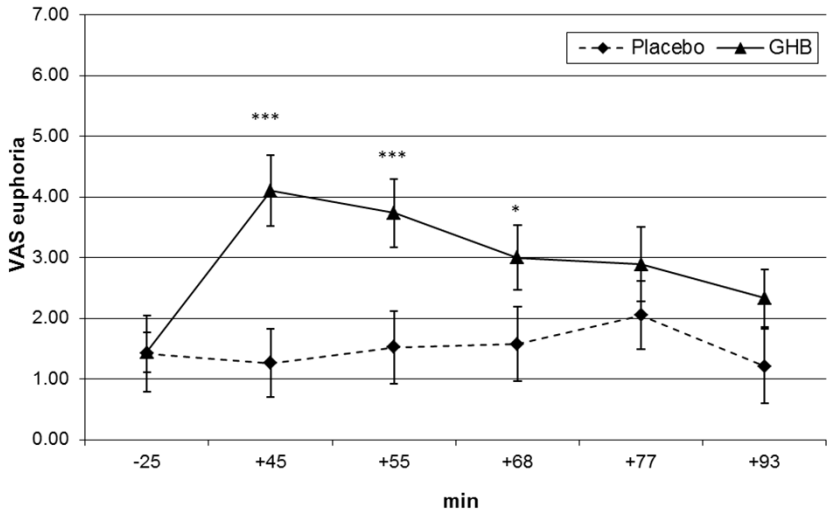
Fig. 2: Right anterior insula seed resting state functional connectivity increases GHB vs. placebo: A) Bilateral superior frontal gyrus, B) right thalamus, p<.05 (cluster-corrected).

Fig. 3: Anterior cingulate cortex seed resting state functional connectivity decreases GHB vs. placebo: A) bilateral paracentral lobule, p<.05 (cluster-corrected).

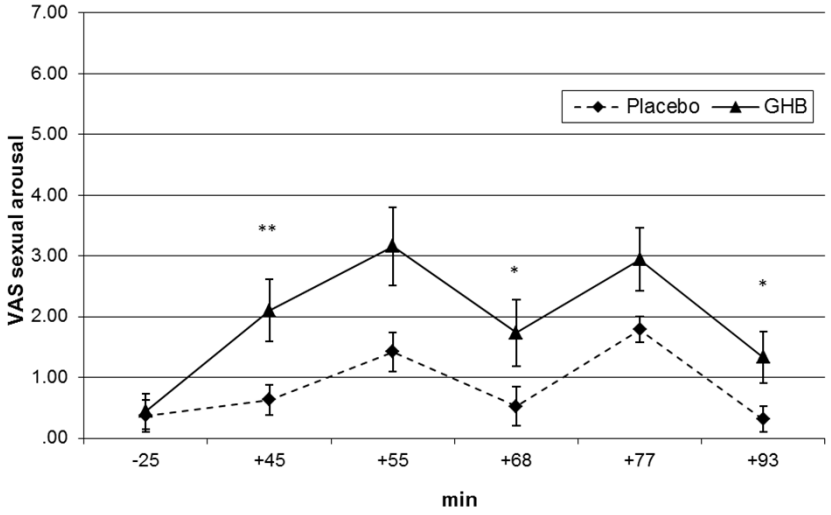
Fig. 4: Correlation of the euphoria visual analogue scale (VAS) scores and increased resting-state functional connectivity of the right anterior insula with the bilateral superior frontal cortex, GHB vs. placebo (p<.05, uncorrected).

FIG. 1

A)


7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
VAS euphoria
Placebo
GHB
***
***
*
-25
+45
+55
+68
+77
+93
min

B)


7.00
6.00
5.00
4.00
3.00
2.00
1.00
.00
VAS sexual arousal
Placebo
GHB
**
*
*
-25
+45
+55
+68
+77
+93
min

C)

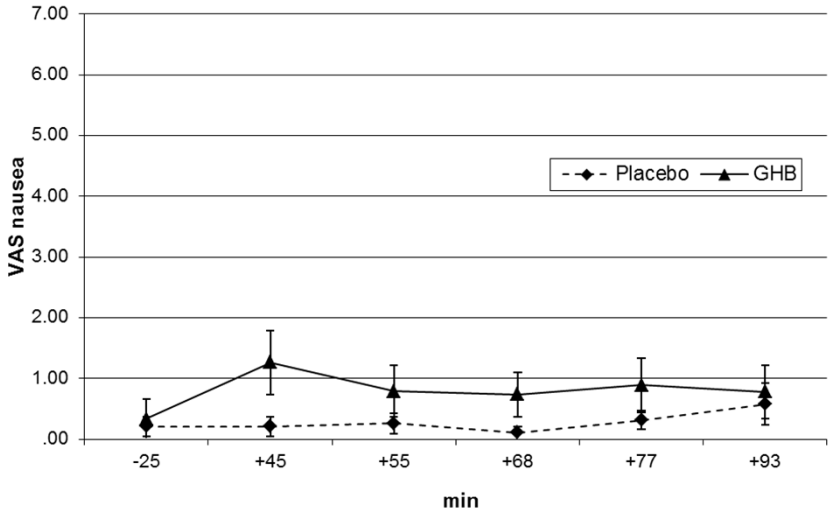
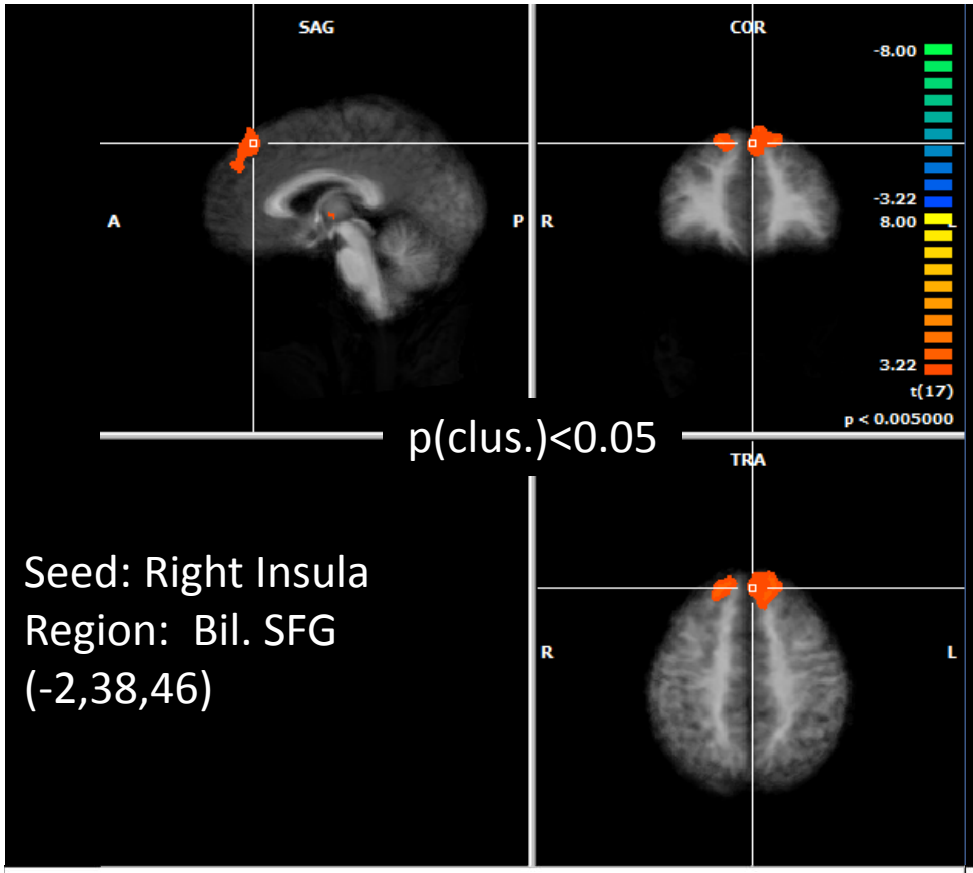
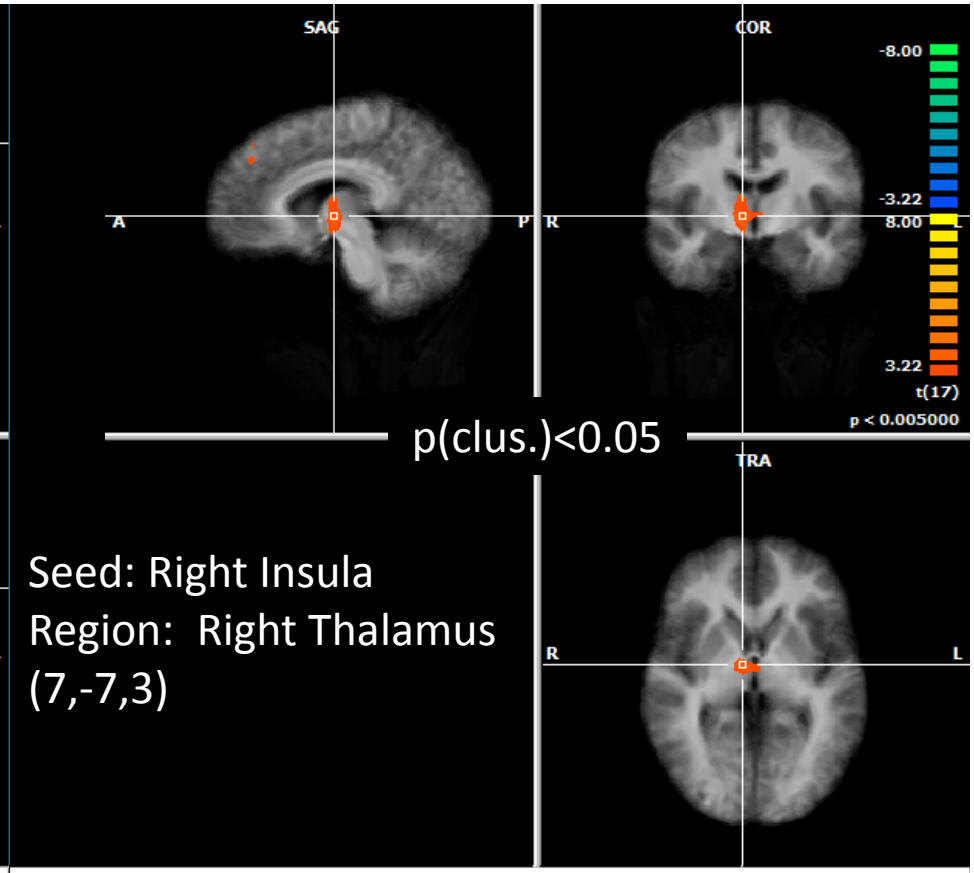
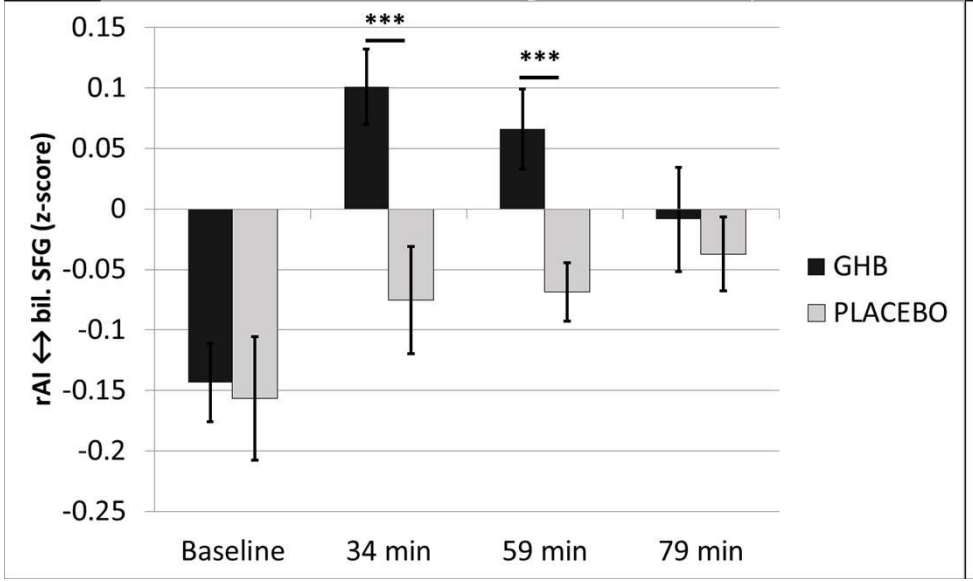

7.00
6.00
5.00
4.00
3.00
2.00
1.00
.00
VAS nausea
Placebo
GHB
-25
+45
+55
+68
+77
+93
min

FIG. 2


SAG
COR
TRA
-8.00
-3.22
8.00
3.22
t(17)
p < 0.005000
A
P
R
L
p(clus.)<0.05
Seed: Right Insula
Region: Bil. SFG
(-2,38,46)


SAG
COR
TRA
-8.00
-3.22
8.00
3.22
t(17)
p < 0.005000
A
P
R
L
p(clus.)<0.05
Seed: Right Insula
Region: Right Thalamus
(7,-7,3)


rAI ↔ bil. SFG (z-score)
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
***
***
Baseline
34 min
59 min
79 min
GHB
PLACEBO

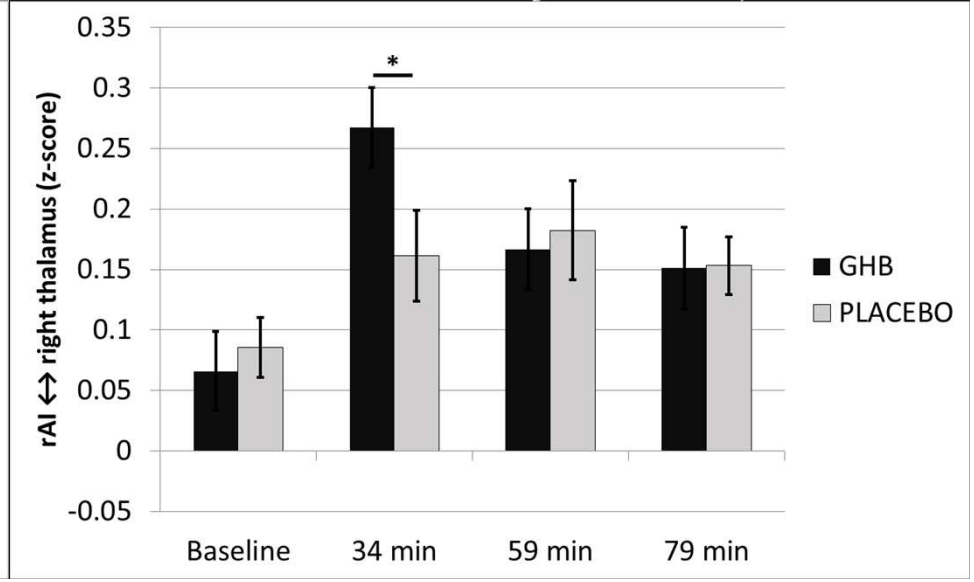

rAI ↔ right thalamus (z-score)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
*
Baseline
34 min
59 min
79 min
GHB
PLACEBO

FIG. 3

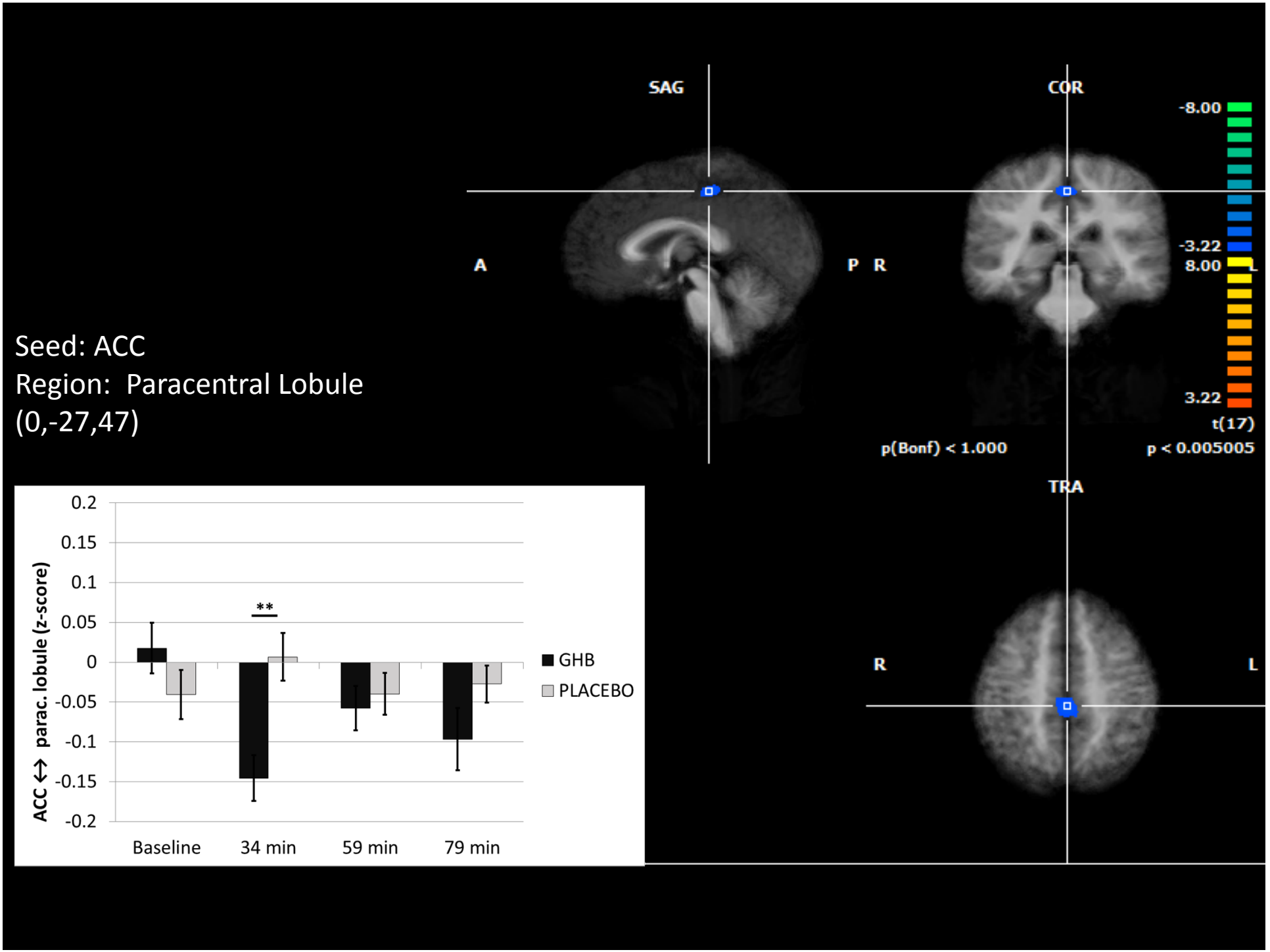
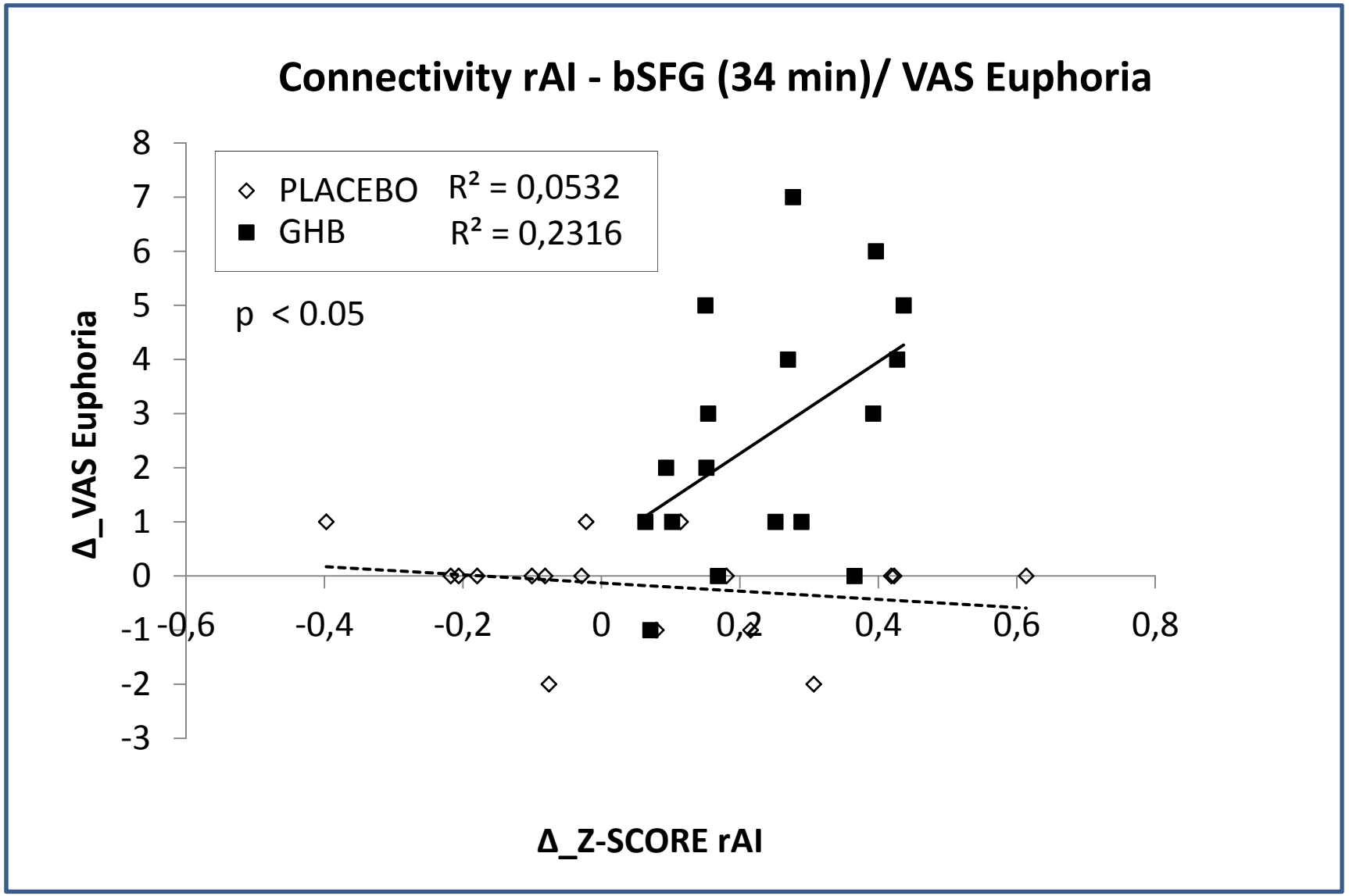

SAG
COR
-8.00
-3.22
8.00
3.22
t(17)
p < 0.005005
A
P
R
L
p(Bonf) < 1.000
TRA
R
L
Seed: ACC
Region: Paracentral Lobule
(0,-27,47)
ACC ↔ parac. lobule (z-score)
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
**
Baseline
34 min
59 min
79 min
GHB
PLACEBO

FIG. 4


Connectivity rAI - bSFG (34 min)/ VAS Euphoria
PLACEBO R² = 0,0532
GHB R² = 0,2316
p < 0.05
Δ_VAS Euphoria
Δ_Z-SCORE rAI
8
7
6
5
4
3
2
1
0
-1
-2
-3
-0,6
-0,4
-0,2
0
0,2
0,4
0,6
0,8